TITLE:

Experimental Study on the Role of Essential Fatty Acids and Pyridoxine on Adrenocortical Function

AUTHOR(S):

MURAOKA, RYUSUKE

CITATION:

MURAOKA, RYUSUKE. Experimental Study on the Role of Essential Fatty Acids and Pyridoxine on Adrenocortical Function. 日本外科宝函 1965, 34(1): 35-54

ISSUE DATE:

1965-01-01

URL:

http://hdl.handle.net/2433/206453

RIGHT:

京都大学学術情報リポジトリ
KURENAI 紅
Kyoto University Research Information Repository

Experimental Study on the Role of Essential Fatty Acids and Pyridoxine on Adrenocortical Function

by

RYUSUKE MURAOKA

From the 2nd Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. CHUJI KIMURA)
Received for Publication Nov. 10, 1964

INTRODUCTION

The high concentration of essential fatty acids (abbreviated as EFA) as well as of cholesterol in the adrenal gland suggests that the metabolism of these acids are related to the formation and secretion of adrenocortical hormones.

MATSUDA in our laboratory has observed that animals deficient in EFA show an earlier exhaustion of liver glycogen during fasting and succumb to starvation sooner than controls. This has been considered to be a reflection of decreased adrenocortical activity. NAGASE has also shown in his study on acute postoperative pulmonary edema that EFA deficiency induces an increase in capillary permeability owing not only to structural changes in the capillary wall but also to decrease in adrenocortical capacity. Subsequently, it has been confirmed by colleague TAMAKI that the adrenals of EFA-deficient rats secrete smaller quantities of steroid hormones *in vivo* under various stresses. Furthermore, colleague ISHIMARU has observed electron microscopically that the adrenocortical fasciculata cells of EFA-deficient animals show a decrease in number of mitochondria and changes in their inner structure : vacuolization, depletion of smooth-surfaced endoplasmic reticulum, disappearance of fine granules, development of gross fat granules, etc.. According to colleague KUMANO the administration of cholesterol to EFA-deficient rats aggravates their EFA-deficiency and decreases adrenocortical capacity in spite of increased total cholesterol and esters in the adrenals.

From all of these observations in our laboratory and the reports of HAYASHIDA and PORTMAN that the adrenals of EFA-deficient rats secrete smaller quantities of steroid hormones *in vitro* under stimulation of ACTH, and of SKOVSTEAD et al., who obtained the same result *in vivo*, it is obvious that EFA in the adrenals is one of the most important factors influencing adrenocortical function. Nevertheless, the functional roles of these acids in the adrenals have not yet been clarified.

From the fact that cholesterol can serve as a precursor of adrenal steroid hormones, and that administration of ACTH to rats produces a marked drop in the cholesterol ester fraction of the adrenal, it has been considered that adrenal cholesterol esters may be involved in steroid hormone biosynthesis.

The present investigation was designed to clarify the role of EFA, and especially that of adrenal cholesterol ester fractions on adrenocortical activity. Pyridoxine has been suggested by many investigators to have an important relationship to lipid metabolism.

Therefore, a study of the effect of pyridoxine on fatty acid metabolism and on adrenocortical activity was included.

EXPERIMENTAL ANIMALS AND METHODS

1) Experimental Animals

Male albino rats of the Wistar strain supplied by the Animal Center of Kyoto University were used for this study. The weanling rats were fed a rat chow (a product of Oriental Yeast Ind. Co. Japan) until their body weight reached about 40-50 g, then were divided randomly into three groups ; a fat diet group, a fat-deficient diet group and a pyridoxine-deficient fat diet group. These groups were maintained on their respective diets listed in Table 1 *ad libitum* for 12 weeks in all experiments except the last group which was fed a pyridoxine-deficient fat diet for 6 weeks after a fat diet for 6 weeks.

As the source of EFA, a purified and peroxide-free sesame oil was used. The fatty acid composition of the sesame oil analyzed by gas-liquid chromatography was as follows ; C16 : 0 9.5%, C16 : 1 0.7%, C18 : 0 5.1%, C18 : 1 34.6%, C18 : 2 48.9%, C18 : 3 0.7 % and C20 : 0 0.5%. Each gram of the casein and the starch used in this study contained respectively, 437 μg and 46 μg of total fatty acids, 7 μg and 7 μg of linoleic acid, 3 μg and 2 μg of linolenic acid and no other polyunsaturated fatty acids (abbreviated as PUFA) according to gas-liquid chromatographic analysis. Therefore, a rat eating 10 g of the fat-deficient diet per day ingests less than 0.1 mg of EFA per day.

Table 1 Composition of the diets

	Fat diet	Fat-deficient diet	Pyridoxine-deficient fat diet
Starch	60%	80%	60%
Casein	16	16	16
Sesame oil	20	0	20
Salt-mixture	3	3	3
Vitamin-mixture	0.5	0.5	0.5 (Pyridoxine free)
Choline chloride	0.5	0.5	0.5

2) Methods

After an overnight fast, animals were anesthetized by intraperitoneal injection of nembutal and blood was collected from the abdominal aorta. Then both adrenals were removed immediately, dissected free of surrounding fat and weighed. The left adrenal from each rat was used for lipid analysis and the right one and serum for hormone determination by colleague FUKUDA. Because of their small size, the left adrenals obtained from the five animals were pooled to provide a single sample.

The pooled adrenals, after homogeni-

Vitamin-mixtuure per 1 g

Vitamin A	2500 I. U.
B_1	1.0mg
B_2	1.5mg
B_6 (Pyridoxine)	1.0mg
B_{12}	1.0γ
C	37.5mg
D	200 I. U.
E	1.0mg
K	0.2mg
Niacin	10.0mg
Pantothenic acid	2.5mg
Folic acid	0.5mg

zation were extracted with ethanol-ether 3 : 1 as described by our colleague JINDO. Cholesterol esters of adrenal lipids were separated from the other lipid components by chromatography on silicic acid according to the method of HIRSCH and AHRENS with minor modifications. A sharp separation of the esters from the other lipid components was obtained. Total lipids and cholesterol esters were then saponified with 2 % ethanolic KOH at 60°C for 1 hour. After extraction of nonsaponifiable materials from the alkaline solution with petroleum ether, the total fatty acids and the cholesterol ester fatty acids were removed by acidification of the aqueous solution and subsequent extraction with petroleum ether. Petroleum ether extract of nonsaponifiable materials was used to determine total cholesterol and esterified cholesterol by the method of ABELL et al..

For fatty acid analysis, two different methods were used; alkaline isomerization for PUFA content and gas-liquid chromatography for fatty acid composition of these materials. PUFA of total lipids and cholesterol esters were determined by ultraviolet spectrophotometry after alkaline isomerization for 20 min. at 180°C, using 21% KOH in ethylene glycol according to the method of JINDO, which is a modification of HOLMAN and HAYES.

The residual fatty acids from total lipids and cholesterol esters were methylated by using 2% methanolic sulfuric acid at 70 C for 90 min.. The methyl esters of the fatty acids were analyzed in a SHIMADZU Gas Chromatograph Model GC 1-B equipped with a hydrogen flame ionization detector. A 150 cm U-shaped stainless-steel column of 6 mm i. d., packed with 25% diethylene glycol succinate coated on Shimalite-Q, 60-80 mesh was used at 210°C. Nitrogen was the carrier gas, and the flow rate was 30ml/min. at an inlet pressure of 3 Kg/cm^2 The inlet heater was kept at 300°C and the detector cell at 235 C. Approximately 5 μl of a solution of the methyl esters in petroleum ether was injected. The individual esters were identified by carbon number and by internal standards wherever feasible. Authentic methyl esters of fatty acids, obtained from the National Institute of Health (U.S.A.) and Hormel Institute (U.S.A.), were used as internal standards. Quantification was carried out by triangulation. The fatty acid composition is reported as area per cent, and the area percent of mixed methyl esters of standard saturated C8-C22 fatty acids well agreed with their weight per cent as reported previously.

RESULTS

I Body weight and adrenal weight

Body weight, adrenal weight and adrenal weight per 100 g body weight of the three diet groups at the end of the feeding period are shown in Table 2.

During the course of the feeding period, all rats receiving a fat-deficient diet exhibited the signs of EFA deficiency; scaly paws and tails, loss of hair on the back, on the neck

Table 2 Body weight, adrenal weight and adrenal weight per 100 g body weight of the three diet groups at the end of the feeding period

Group	Body weight	Adrenal weight	Adrenal weight per 100g body weight
Fat diet	250±16.8 g*	18.7±1.1mg	7.8±0.6mg
Fat-deficient diet	145±5.9	15.3±0.8	10.7±0.6
Pyridoxine-deficient fat diet	116±11.2	17.9±1.4	12.4±0.7

* Standard error of the mean.

and around the face, and stunted growth. In the rats fed a pyridoxine-deficient fat diet, mild dermatitis and retardation of growth were observed.

Adrenal weight per 100 g body weight of fat-deficient rats or pyridoxine-deficient rats was significantly greater than that of rats fed supplemented fat diet. Body weight, and adrenal weight per 100 g body weight of the rats fed pyridoxine-deficient fat diet were similar to those of fat-deficient rats, and there were no significant differences between them.

II Adrenal PUFA content, fatty acid composition and cholesterol content

A) Resting state

1) Adrenal PUFA content

Table 3 shows the adrenal PUFA content of total lipids and cholesterol ester fractions of the three diet groups analyzed by alkaline isomerization. The adrenals of the rats fed a fat-deficient diet showed decrease in dienoic and tetraenoic acids which were abundant in the adrenals of the rats fed a fat diet or a pyridoxine-deficient fat diet. They showed an increase in trienoic acid, of which there was little in the adrenals of the last two groups. Tetraenoic acid content of the rats fed a pyridoxine-deficient fat diet was as high or higher than those of supplemented animals. However, dienoic acid content was lower in the adrenals of the rats fed a pyridoxine-deficient fat diet than in those supplemented. In all groups, the greater part of PUFA except dienoic acid occurred in their cholesterol ester fractions.

2) Fatty acid composition of adrenal lipids

Since alkaline isomerization technique did not distinguish between PUFA which had the same number of double bonds but which differed in chain length, gas-liquid chromatography was carried out to determine individual fatty acids.

The fatty acid composition of adrenal total lipids and cholesterol esters of the three diet groups as analyzed by gas-liquid chromatography is shown in Table 4. Adrenal total lipids of EFA supplemented animals (fat diet group and pyridoxine-deficient fat diet group) exhibited high proportions of 18 : 2, 20 : 4 acids and low of 16 : 1, 18 : 1, 20 : 3 and 22 : 3 acids. Contrary, EFA-deficient group showed high proportions of 16 : 1, 18 : 1, 20 : 3 and 22 : 3 acids and low of 18 : 2, 20 : 4 and 22 : 4 acids. The fatty acid com-

Table 3 Adrenal PUFA content of total lipids and cholesterol esters of the three diet groups

Group	No. of rats		Adrenal PUFA (mg/100mg adrenal)				
			Di	Tr	Tt	Pt	Hx
Fat diet	5	Total PUFA	1.10	0.17	1.55	0.14	0.03
		Ch-ester PUFA	0.13	0.12	1.07	0.11	0.03
Fat-deficient diet	5	Total PUFA	0.19	1.51	0.86	0.17	0.10
		Ch-ester PUFA	0.12	0.96	0.70	0.15	0.09
Pyridoxine-deficient fat diet	5	Total PUFA	0.45	0.16	1.79	0.18	0.04
		Ch-ester PUFA	0.13	0.11	1.51	0.15	0.03

Di : dienoic acid Tr : trienoic acid Tt : tetraenoic acid Pt : pentaenoic acid Hx : Hexaenoic acid

Table 4 The fatty acid composition of adrenal total lipids and cholesterol esters of the three diet groups

Fatty acid	Fat diet group		Fat-deficient diet group		Pyridoxine-deficient fat diet group	
	Total	Ch-ester	Total	Ch-ester	Total	Ch-ester
14 : 0	1.2	1.4	1.6	1.9	1.0	0.7
16 : 0	15.5	9.6	17.0	11.7	12.3	8.7
16 : 1	1.4	2.5	5.9	5.3	1.7	0.8
18 : 0	1.5	1.0	3.1	1.4	2.0	1.2
18 : 1	34.3	18.3	31.6	18.8	22.3	13.8
18 : 2	15.0	5.3	2.4	1.8	9.8	1.7
18 : 3	0.2	0.9			0.7	
20 1	1.8	6.2	1.7	7.0	3.9	5.6
20 : 3	0.2	3.5	7.7	11.0	1.4	2.6
20 : 4	11.2	19.0	1.8	7.1	22.7	24.6
22 : 0	0.5	1.6	1.0	1.7	0.6	1.4
22 : 3	0.7	1.4	7.7	13.8		0.7
22 : 4	11.1	24.5	7.1	13.1	19.4	29.1
22 : 5	1.5	2.3	2.0	1.9	1.0	2.6
22 : 6	1.1	1.1	2.2	1.8	0.7	2.3

5 rats in each group.

position of the adrenal cholesterol esters of the three diet groups showed differences similar to those of total lipids indicated above, but C16 and C18 acid proportions were smaller and C20 and C22 acids were greater than that of total lipids.

According to MOHRHAUER and HOLMAN, eicosatetraenoic acid (20 : 4) from rat liver lipids consists mainly of arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid) and small amounts of 4, 7, 10, 13-eicosatetraenoic acid. The eicosatrienoic acid (20 : 3) in liver lipids of fat-deficient rats consists mainly of 5, 8, 11-eicosatrienoic acid with small amounts of the 7, 10, 13-isomer as determined by reductive ozonolysis. Table 5 shows metabolic pathways of PUFA in animal bodies as assumed in our laboratory largely based upon the works of MEAD and HOLMAN and their coworkers. From this schema, it is obvious that increased fatty acids in fat-deficient animals are those of the endogenously derived oleic and palmitoleic acid families. However, in EFA supplemented animals, an increase is found in linoleic acid which MEAD et al. reported can never be synthesized in animal bodies, and in fatty acids of linoleic acid family.

Table 5 Metabolic pathways of polyunsaturated fatty acids in animal bodies

1) $CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$
Linoleic acid (9, 12-Octadecadienoic acid) C_{18}, $\Delta_{9,12}$ (18 : 2ω6)
↓ −2H
γ-Linolenic acid (6, 9, 12-Octadecatrienoic acid) C_{18}, $\Delta_{6,9,12}$ (18 : 3ω6)
↓ +2C
8, 11, 14-Eicosatrienoic acid C_{20}, $\Delta_{8,11,14}$ (20 : 3ω6)
↓ −2H
Arachidonic acid (5, 8, 11, 14-Eicosatetraenoic acid) C_{20}, $\Delta_{5,8,11,14}$ (20 : 4ω6)
↓ +2C
7, 10, 13, 16-Docosatetraenoic acid C_{22}, $\Delta_{7,10,13,16}$
↓ −2H
4, 7, 10, 13, 16-Docosapentaenoic acid C_{22}, $\Delta_{4,7,10,13,16}$ (22 : 5ω6)

2) $CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$
Linolenic acid (9, 12, 15-Octadecatrienoic acid) C_{18}, $\Delta_{9,12,15}$ (18 : 3ω3)
↓ −2H
6, 9, 12, 15-Octadecatetraenoic acid C_{18}, $\Delta_{6,9,12,15}$
↓ +2C
8, 11, 14, 17-Eicosatetraenoic acid C_{20}, $\Delta_{8,11,14,17}$ (20 : 4ω3)
↓ −2H
5, 8, 11, 14, 17-Eicosapentaenoic acid C_{20}, $\Delta_{5,8,11,14,17}$
↓ +2C
7, 10, 13, 16, 19-Docosapentaenoic acid C_{22}, $\Delta_{7,10,13,16,19}$ (22 : 5ω3)
↓ −2H
4, 7, 10, 13, 16, 19-Docosahexaenoic acid C_{22}, $\Delta_{4,7,10,13,16,19}$

3) $CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH$
Oleic acid (9-Octadecenoic acid) C_{18}, Δ_9
↓ −2H
6, 9-Octadecadienoic acid C_{18}, $\Delta_{6,9}$ (18 : 2ω9)
↓ +2C
8, 11-Eicosadienoic acid C_{20}, $\Delta_{8,11}$
↓ −2H
5, 8, 11-Eicosatrienoic acid C_{20}, $\Delta_{5,8,11}$ (20 : 3ω9)
↓ +2C
7, 10, 13-Docosatrienoic acid C_{22}, $\Delta_{7,10,13}$

4) $CH_3-(CH_2)_5-CH=CH-(CH_2)_7-COOH$
Palmitoleic acid (9-Hexadecenoic acid) C_{16}, Δ_9
↙ −2H ↘ +2C
6, 9-Hexadecadienoic acid C_{16}, $\Delta_{6,9}$ Vaccenic acid (11-Octadecenoic acid) C_{18}, Δ_{11}
↘ +2C ↙ −2H
8, 11-Octadecadienoic acid C_{18}, $\Delta_{8,11}$ (18 : 2ω7)
↓ −2H
5, 8, 11-Octadecatrienoic acid C_{18}, $\Delta_{5,8,11}$ (18 : 3ω7)
↓ +2C
7, 10, 13-Eicosatrienoic acid C_{20}, $\Delta_{7,10,13}$ (20 : 3ω7)
↓ −2H
4, 7, 10, 13-Eicosatetraenoic acid C_{20}, $\Delta_{4,7,10,13}$ (20 : 4ω7)

From the results obtained by alkaline isomerization and gas-liquid chromatography, EFA, especially arachidonic acid which generally is considered to be the most important one, were contained largely in cholesterol esters in all groups.

3) Adrenal cholesterol content

The values for total and esterified cholesterol in adrenals of the three diet groups are shown in Table 6. The increase in total and esterified cholesterol in the EFA-deficient animals is in agreement with the reports of ALFIN-SLATER et al. and HAYASHIDA and PORTMAN. It is particularly interesting that adrenals of EFA-deficient rats have an increased cholesterol which is considered an important precursor of adrenocortical hormones,

Table 6 Adrenal cholesterol and corticosterone content and serum corticosterone levels of the three diet groups

Group	No. of rats	Adrenal cholesterol mg/100mg adr.		Adrenal corticosterone*		Serum corticosterone*
		Total	Esterified	μg/100mg adr.	μg/adr.	μg/100ml
Fat diet	5	5.67	4.96	3.5	0.84	40
Fat-deficient diet	5	8.22	7.70	2.9	0.51	25
Pyridoxine-deficient fat diet	5	5.72	5.07	3.3	0.60	36

* Measured by colleague Fukuda.

but have a reduced adrenocortical secretion as shown by FUKUDA.

B) A single administration of ACTH

1) Adrenal PUFA content

Table 7 shows the changes in adrenal PUFA content of the three diet groups following a single administration of ACTH-Z in a dose of 3 I. U. into the back muscles. All PUFA in total lipids and in cholesterol esters decreased following injection of ACTH in each group.

2) Fatty acid composition of adrenal lipids

Besides the three diet groups described above, a newly prepared fat diet group and a fat-deficient diet group were analyzed by gas-liquid chromatography.

As shown in Table 8, area percent of 20 : 4 acid decreased in cholesterol esters following ACTH injection in all groups, while serum corticosterone levels rose and reached their maximum after 1-2 hours following ACTH injection as shown by FUKUDA. The

Table 7 Changes in adrenal PUFA content of the three diet groups following a single administration of ACTH-Z 3 I. U. (mg/100mg adrenal)

Fat diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	1.10	0.17	1.55	0.14	0.03	0.13	0.12	1.07	0.11	0.03
1	5	0.72	0.15	0.99	0.12	0.03	0.11	0.08	0.68	0.10	0.02
2	5	0.73	0.15	0.94	0.11	0.03	0.11	0.08	0.75	0.09	0.02
4	5	0.78	0.14	1.15	0.10	0.02	0.12	0.08	0.80	0.07	0.03
6	5	0.69	0.10	1.02	0.10	0.02	0.08	0.05	0.55	0.07	0.02
12 hrs.	5	0.80	0.18	1.44	0.12	0.02	0.12	0.11	1.05	0.09	0.02

Fat-deficient diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.19	1.51	0.86	0.17	0.10	0.12	0.96	0.70	0.15	0.09
1	5	0.18	1.55	0.84	0.18	0.08	0.09	0.97	0.68	0.15	0.07
2	5	0.16	1.02	0.75	0.14	0.11	0.07	0.95	0.63	0.13	0.10
4	5	0.12	1.09	0.45	0.12	0.06	0.08	0.59	0.33	0.10	0.06
6	5	0.16	1.05	0.70	0.15	0.06	0.09	0.77	0.52	0.13	0.05
12 hrs.	5	0.09	0.42	0.29	0.06	0.02	0.05	0.28	0.13	0.04	0.02

Pyridoxine-deficient fat diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.45	0.16	1.79	0.18	0.04	0.13	0.11	1.51	0.15	0.03
2	5	0.36	0.15	1.38	0.16	0.03	0.09	0.10	0.87	0.13	0.02
4	5	0.37	0.13	0.67	0.10	0.02	0.04	0.08	0.37	0.07	0.02
6 hrs.	5	0.51	0.11	1.05	0.17	0.03	0.07	0.08	0.64	0.13	0.02

corticosterone response in serum after ACTH stimulation was markedly reduced in the rats fed fat-deficient diet. But the difference between the rats fed supplemented fat diet

Table 8 Changes in the fatty acid composition of adrenal cholesterol esters following a single administration of ACTH-Z 3 I. U.

Fatty acid	Fat diet group No. 1				Fat diet group No. 2				Fat-deficient diet group No. 1				Fat-deficient diet group No. 2				Pyridoxine-deficient fat diet group			
	Before	2	4	6	Before	2	4	6	Before	2	4	6	Before	2	4	6	Before	2	4	6 hrs.
14 : 0	1.4	1.6	1.6	1.1	1.3	1.5	1.6	1.4	1.9	1.4	1.7	2.0	2.2	1.7	2.1	1.7	0.7	1.1	1.5	1.0
16 : 0	9.6	11.5	11.0	11.3	9.5	9.9	10.8	9.5	11.7	15.7	12.6	21.5	11.2	10.9	10.6	10.2	8.7	10.8	15.1	12.6
16 : 1	2.5	2.6	2.5	2.1	2.6	2.1	2.6	2.2	5.3	5.0	4.8	5.8	6.0	5.5	6.2	5.0	0.8	1.4	1.5	1.5
18 : 0	1.0	1.1	0.8	0.9	1.1	1.0	1.2	1.1	1.4	1.3	1.3	2.0	1.5	1.4	1.4	1.3	1.2	1.0	1.1	1.2
18 : 1	18.3	18.7	14.6	14.8	18.1	14.9	15.3	14.4	18.8	18.5	11.7	17.1	18.1	16.8	15.3	15.8	13.8	12.3	12.9	14.0
18 : 2	5.3	5.7	4.8	5.1	1.4	3.7	3.8	3.2	1.8	1.7	1.8	1.8	2.2	1.9	2.1	2.0	1.7	5.0	4.4	5.3
18 : 3	0.9	1.6	2.7	2.5	0.3	0.2	0.2	0.1												
20 : 1	6.2	7.8	7.6	5.9	1.3	4.9	5.6	5.5	7.0	6.4	5.7	5.1	8.1	8.0	8.8	7.8	5.6	4.3	6.1	4.9
20 : 3	3.5	3.0	2.8	3.0	3.0	2.8	2.2	3.8	11.0	10.8	12.0	6.2	9.6	12.0	7.1	10.0	2.6	2.8	1.5	1.7
20 : 4	19.0	12.8	13.6	13.8	20.0	15.8	11.7	14.6	7.1	5.4	6.2	3.5	1.7	1.1	3.7	4.4	24.6	19.1	13.5	19.6
22 : 0	1.6	2.8	3.5	3.2	1.4	1.9	2.2	1.7	1.7	1.1	1.5	2.1	2.3	1.9	3.3	2.1	1.4	1.2	2.7	1.2
22 : 3	1.4	1.2	1.6	1.1	1.2	1.5	2.2	1.5	13.8	15.7	25.0	16.7	13.9	16.5	14.8	17.7	0.7	0.6	1.0	0.5
22 : 4	24.5	25.1	27.2	29.3	24.9	29.7	30.6	28.4	13.1	13.4	10.8	12.5	13.4	13.3	16.4	14.4	29.4	33.5	31.1	28.6
22 : 5	2.3	2.6	2.5	2.7	5.2	6.3	4.4	5.6	1.9	2.5	2.4	1.7	3.1	2.9	4.4	3.6	2.6	3.6	1.1	1.4
22 : 6	1.1	0.7	1.3	1.2	2.2	3.2	3.9	4.0	1.8	2.0	2.0	1.5	3.1	2.5	2.5	3.5	2.3	2.1	1.6	2.1

5 rats in each group.

Table 9 Ratio changes in cholesterol ester fatty acids after a single administration of ACTH-Z 3 I. U.

Fatty acid	Before	2 hrs. after ACTH injection	Significant change p=0.05
14 : 0	1.00	1.07±0.16*	–
16 : 0	1.00	1.16±0.08	
16 : 1	1.00	1.11±0.17	–
18 : 0	1.00	0.94±0.11	–
18 : 1	1.00	0.92±0.06	–
18 : 2	1.00	0.95±0.05	–
20 : 1	1.00	1.02±0.09	–
20 : 3	1.00	1.02±0.08	–
20 : 4	1.00	0.77±0.04	+ ($p<0.01$)
22 : 0	1.00	1.09±0.23	–
22 : 3	1.00	1.06±0.09	–
22 : 4	1.00	1.07±0.05	–
22 : 5	1.00	1.19±0.11	–
22 : 6	1.00	0.98±0.16	–

* Standard error of the mean.

and pyridoxine-deficient fat diet was not distinct following a single administration of ACTH (Fig. 1). Two hours after the injection, 20 : 4 acid in cholesterol esters decreased significantly (p 0.01), but the fatty acids other than 20 : 4 acid did not show any significant change ($p>0.05$) (Table 9). Fig. 2 shows changes in adrenal arachidonic acid content in each group calculated from tetraenoic acid content by alkaline isomerization, and the ratio of 20 : 4 and 22 : 4 acids by gas-liquid chromatography following a single administration of ACTH.

3) Adrenal cholesterol content

In all groups, parallel decrease in adrenal total and esterified cholesterol following ACTH injection was observed (Fig. 3). These data show that most of these changes occur in the ester fraction.

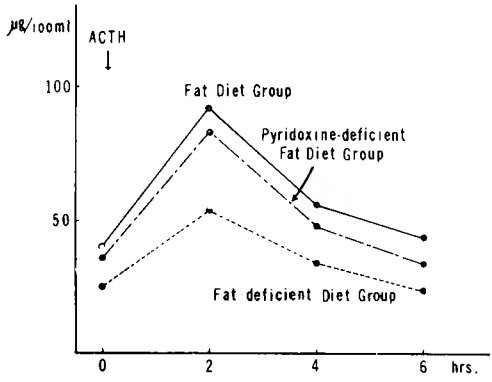

Fig. 1 Changes in serum corticosterone levels of the three diet groups following a single administration of ACTH-Z 3 I. U. (*Fudauk*)

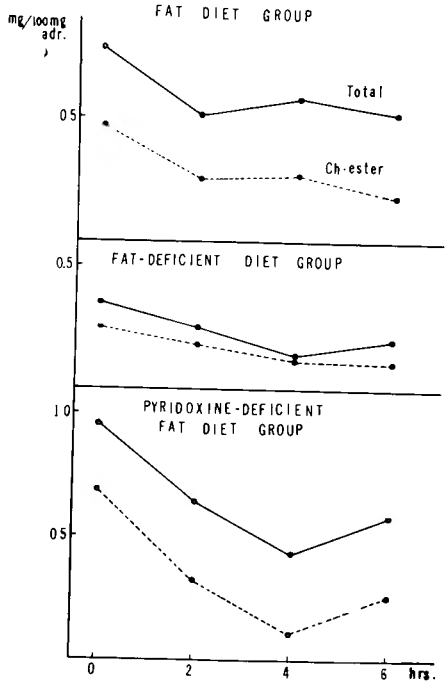

Fig. 2 Changes in adrenal arachidonic acid content of the three diet groups following a single administration of ACTH-Z 3 I. U.

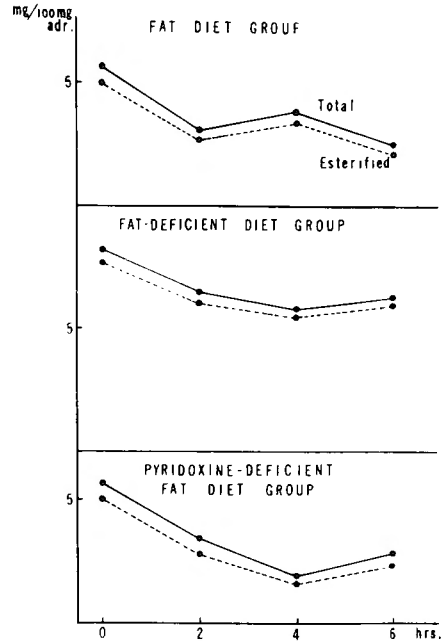

Fig. 3 Changes in adrenal cholesterol content of the three diet groups following a single administration of ACTH-Z 3 I. U.

C) Successive administration of ACTH for 4 days

Three diet groups of the rats were injected with ACTH-Z in a dose of 3 I. U into back muscles on 4 successive days. In the course of this experiment, animals were fed respective diets *ad libitum*, but an overnight fast was forced before sacrifice. Two hours after the injection on the second and fourth day, rats fed fat diet or fat-deficient diet were sacrificed and adrenal lipids were analyzed. As for the rats on pyridoxine-deficient fat diet the procedure was carried out on the fourth day only.

Adrenal PUFA content and fatty acid composition during successive ACTH injection are represented in Table 10 and 11. Fig. 4 and 5 show changes in adrenal arachidonic acid and cholesterol content during successive ACTH injection. The first day's values are borrowed from that of 2 hours after a single administration of ACTH in previous experiment.

Rats fed fat diet contained arachidonic acid abundantly in adrenals both in total lipids and in cholesterol esters from the first day through the fourth day, but in rats fed fat-

Table 10 Changes in adrenal PUFA content of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days (mg/100mg adrenal)

Fat diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.87	0.13	1.43	0.13	0.04	0.14	0.08	0.95	0.10	0.03
2	5	0.61	0.09	1.04	0.10	0.03	0.10	0.05	0.50	0.06	0.02
4 days	5	0.52	0.10	1.00	0.09	0.04	0.08	0.07	0.59	0.06	0.03

Fat-deficient diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.13	0.75	0.81	0.13	0.09	0.08	0.62	0.57	0.10	0.08
2	5	0.14	0.73	0.76	0.12	0.08	0.07	0.56	0.47	0.10	0.07
4 days	5	0.57	0.56	0.44	0.07	0.06	0.03	0.23	0.20	0.06	0.04

Pyridoxine-deficient fat diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.45	0.16	1.79	0.18	0.04	0.13	0.11	1.51	0.15	0.04
4 days	5	0.50	0.09	0.98	0.07	0.02	0.04	0.04	0.33	0.04	0.01

Table 11 Changes in the fatty acid composition of adrenal cholesterol esters of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days

Fatty acid	Fat diet group			Fat-deficient diet group			Pyridoxine-deficient fat diet group	
	Before	2	4	Before	2	4	Before	4 days
14 : 0	0.9	0.9	1.0	1.7	2.0	2.3	0.7	1.1
16 : 0	9.6	12.1	10.5	11.6	11.8	15.2	8.7	13.3
16 : 1	0.9	0.7	0.8	3.7	4.2	1.2	0.8	1.4
18 . 0	1.4	1.5	1.2	1.6	1.3	1.1	1.2	1.1
18 : 1	11.4	17.1	11.7	18.8	15.5	15.7	13.8	11.3
18 : 2	6.5	6.8	6.3	tr	tr	tr	1.7	6.0
20 : 1	1.9	5.6	6.3	7.2	7.1	7.1	5.6	3.9
20 3	3.5	1.6	2.5	10.6	10.2	8.4	2.6	2.7
20 : 4	23.1	19.0	18.7	7.6	6.3	5.1	24.6	21.6
22 : 0	1.0	2.1	1.9	1.8	2.0	1.8	1.4	1.4
22 : 3	0.7	0.8	1.0	14.3	16.7	15.2	0.7	0.7
22 : 1	26.4	26.0	30.2	14.7	15.6	11.9	29.1	28.0
22 : 5	2.0	1.9	1.4	1.7	2.1	2.3	2.6	3.0
22 : 6	3.6	3.4	3.6	2.7	2.6	3.3	2.3	0.9

5 rats in each group.

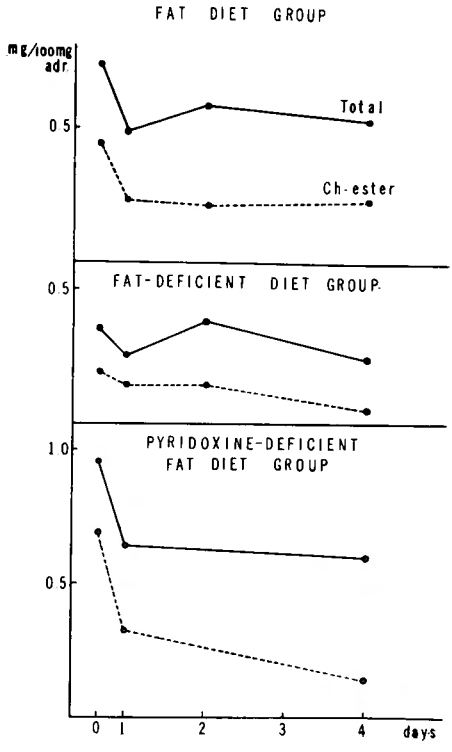

Fig. 4 Changes in adrenal arachidonic acid content of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days

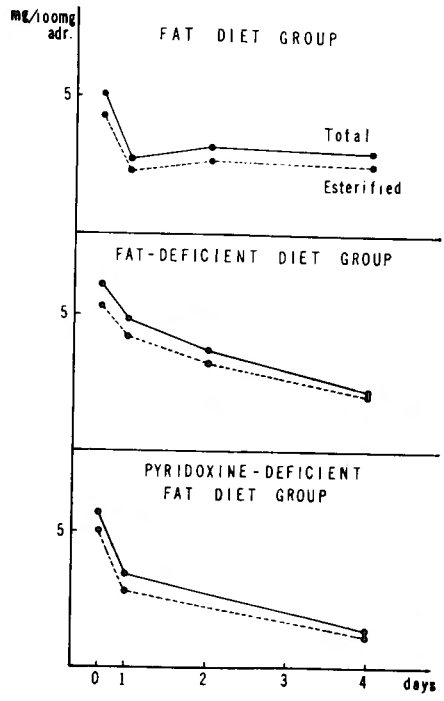

Fig. 5 Changes in adrenal cholesterol content of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days

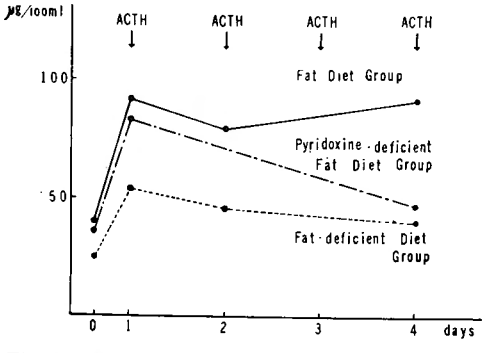

Fig. 6 Changes in serum corticosterone levels of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days (*Fukuda*)

deficient diet adrenal arachidonic acid, already only a small amount at rest, decreased markedly after 4 days, especially in the cholesterol esters. But in rats fed pyridoxine-deficient fat diet, adrenal arachidonic acid in cholesterol esters decreased markedly after 4 days in spite of unchanged level of total arachidonic acid.

According to FUKUDA, changes in serum corticosterone levels during successive ACTH injections are as shown in Fig. 6. Adrenal arachidonic acid content in cholesterol esters changed parallel with serum corticosterone levels during successive ACTH injections (Fig. 7).

Changes in adrenal cholesterol content as illustrated in Fig. 5 had in general the same tendency as those of arachidonic acid, but in all three groups esterified cholesterol changed parallel with total cholesterol, and cholesterol content of the adrenals of rats fed fat-deficient diet in total or in esterified form was high in contrast with their arachidonic acid content.

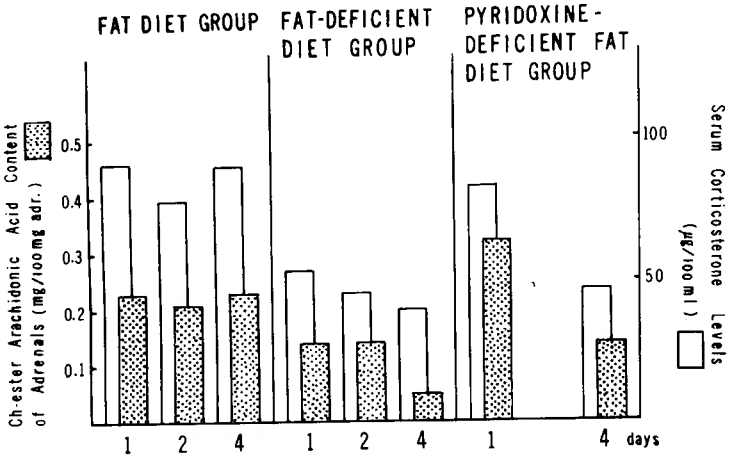

Fig. 7 Serum corticosterone levels and arachidonic acid content in adrenal cholesterol esters of the three diet groups during daily administration of ACTH-Z 3 I. U. for 4 days

APPENDIX : Lipid analyses of 5 human adrenals

1) Materials

As shown in Table 12, 5 human adrenals obtained by necropsy or operation were used in this study.

2) Methods

Extraction, silicic acid chromatography for separation of cholesterol esters and saponification were carried out as described above. After saponification of total lipids and cholesterol ester fractions, an aliquot of the unsaponifiable fraction was used for cholesterol determination as described above. The total fatty acids and the cholesterol ester fatty acids were obtained by acidification of the aqueous solution and subsequent extraction with petroleum ether. The solvent was evaporated to dryness under a flow of nitrogen, and the total fatty acids and the cholesterol ester fatty acids were weighed. Fatty acid preparations were methylated and analysis of the mixtures of fatty acid esters was carried out with gas-liquid chromatography as described above.

3) Results

The results of total cholesterol, esterified cholesterol, total fatty acid and cholesterol ester fatty acid determinations are shown in Table 13, and the fatty acid composition of total lipids and cholesterol esters is in Table 14.

Table 12 Human adrenal gland sources

Case	Age	Sex	Diagnosis	Operation	Cause of death
No. 1	5	Female	Pentalogy of Fallot	Radical operation	Intrathoracal bleeding
2	41	Female	Embolus of common iliac artery	Embolectomy	Uremia
3*	41	Female	Primary aldosteronism	Adrenalectomy (L)	alive
4*	43	Female	Primary aldosteronism	Adrenalectomy (L)	alive
5*	29	Female	Primary aldosteronism	Adrenalectomy (L)	alive

* A normal appearing portion of adrenals was used in this analysis.

Table 13 Cholesterol and fatty acid content of 5 human adrenals

	1	2	3	4	5
Total cholesterol	26.5mg/g	47.0	38.2	40.2	32.1
Esterified cholesterol	11.5	38.3	29.8	37.2	26.2
Total fatty acids	15.0mg/g	30.3	31.9	13.1	28.9
Cholesterol ester fatty acids	7.2	17.7	16.3	8.6	8.7

Table 14 The fatty acid composition of 5 human adrenals

Fatty acid	Total					Cholesterol esters				
	1	2	3	4	5	1	2	3	4	5
14 : 0	1.7	1.7	2.1	1.6	2.3	2.1	1.8	1.4	1.9	1.6
16 : 0	16.5	15.2	19.2	8.2	15.7	7.9	8.6	7.7	8.7	8.3
16 : 1	3.1	5.3	7.0	3.6	6.2	1.3	6.9	5.9	4.2	1.7
18 : 0	7.0	4.2	4.3	2.6	3.5	2.6	2.0	2.2	2.4	2.2
18 : 1	31.6	36.4	43.2	31.4	36.0	31.2	38.4	43.4	34.4	35.1
18 : 2	10.5	10.4	5.4	10.1	11.2	8.7	7.7	3.7	10.3	8.6
18 . 3	0.3	0.5		0.3	0.3	0.3	0.3	0.1	0.1	0.2
20 : 1	2.0	2.3	1.7	2.0	1.0	1.9	2.3	2.8	1.9	1.6
20 : 3	6.9	6.2	3.2	9.3	6.0	9.1	8.7	6.4	10.5	11.0
20 : 4	4.3	3.0	3.2	7.8	4.9	4.3	3.0	4.5	5.3	4.4
22 : 0	1.1	1.3	0.6	0.9	0.5	1.4	1.5	1.1	1.3	1.4
22 : 3	1.1	1.0	0.4	0.9	0.3	2.5	1.5	1.3	0.9	1.3
22 : 4	6.4	3.6	3.3	9.4	1.7	10.5	5.3	7.5	7.7	8.3
22 : 5	0.6	0.2	0.5	1.1	0.5	1.4	0.5	0.6	0.6	1.0
24 : 0	1.2	1.8	0.7	3.4	1.4	1.8	2.4	1.3	2.4	2.6
22 : 6	2.9	2.7	2.0	4.0	2.9	6.3	5.1	3.9	3.3	3.8

The predominant fatty acid was oleic acid. There was a relatively high proportion of 20 : 3 acid and low of 20 : 4 acid, especially in cholesterol esters as compared with rat adrenals. These data are generally in agreement with the report of EBERHAGEN et al.. However, 22 : 6 acid was detected in this study and identified by comparison with lipids from rat brain which contained an extremely high content of 22 : 6 acid.

DISCUSSION

No other organ of the body contains such a high concentration of EFA as the adrenal. Recent investigations in this laboratory have shown that animals deficient in EFA contained low concentrations of dienoic and tetraenoic acids and high concentration of trienoic acid in adrenals, and they exhibited a reduced adrenocortical activity under various stresses, during fasting or even in the resting state. However, little work has been done in determining the fatty acid composition of adrenal lipids concerned with the adrenocortical secretory activity.

Present study revealed that the adrenals of EFA-deficient rats had lower levels of dienoic and tetraenoic acids and higher level of trienoic acid both in total lipids and in cholesterol ester fractions than those of supplemented controls, and FUKUDA showed that

in these animals the secretion of corticosterone by adrenal cortex following administration of ACTH *in vivo* was reduced significantly. Moreover, it was ascertained by gas-liquid chromatography that trienoic acid accumulated in EFA-deficient animals consisted of 20 : 3 and 22 : 3 acids. Which are considered to be 5, 8, 11-eicosatrienoic acid and 7, 10, 13-docosatrienoic acid respectively, derived from oleic acid. And tetraenoic acid which occurred abundantly in the adrenals of EFA supplemented animals consisted of 20 : 4 and 22 : 4 acids, considered to be 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid) and 7, 10, 13, 16-docosatetraenoic acid respectively, both derived from linoleic acid.

These data are generally in accord with those of HAYASHIDA and PORTMAN, and SKOVSTED et al.. However, they analyzed adrenal lipids by the method of alkaline isomerization and not by gas-liquid chromatography, so that the chain length of the acids was not determined.

With respect to the cholesterol, it is well known that adrenal tissue contains extremely high concentrations of cholesterol, and an injection of ACTH depletes the cholesterol content of the adrenal cortex. From these phenomena, together with the fact that cholesterol is closely related chemically to the cortical steroids, it has been suggested by many investigators that cholesterol might be a precursor of the cortical hormones. Direct evidence was obtained by ZAFFARONI et al. in 1951, using cholesterol-C^{14} that adrenal cholesterol was a precursor of cortisol and corticosterone.

However, the present study shows that the adrenal cholesterol content of EFA-deficient rats is higher than that of supplemented ones in spite of their reduced adrenocortical capacity. It is obvious that adrenal cholesterol content by itself is not an index of adrenocortical capacity.

Accordingly, it seems likely from the fact that adrenal cholesterol esters contain a high proportion of EFA, and loss of cholesterol following injection of ACTH is almost entirely due to a decrease in the ester fractions, that adrenal cholesterol esters may be involved in steroid hormone biosynthesis and EFA in cholesterol esters play an important role in formation of steroid hormones. It is particularly interesting to speculate whether specific cholesterol esters are concerned in steroid hormone biosynthesis.

The present experiments showed the proportion of arachidonic acid in adrenal cholesterol esters analyzed by gas-liquid chromatography to be decreased significantly ($p < 0.01$) in the course of 2 hours following administration of ACTH when the corticosterone response in serum was at its maximum as demonstrated by FUKUDA. On the other hand, the proportions of the other fatty acids in adrenal cholesterol esters did not show any significant changes ($p > 0.05$). These results indicate that the cholesterol esterified with arachidonic acid (cholesteryl arachidonate) decreases specifically following administration of ACTH. Furthermore, the serum corticosterone levels changed parallel with the adrenal arachidonic acid content of the cholesterol esters, rather than that of total lipids during successive administration of ACTH.

On the basis of the results presented here, it may be concluded that cholesterol esterified with arachidonic acid is a precursor of steroid hormones in rats. However it is not apparent under the present experimental conditions whether cholesteryl arachidonate is the sole precursor of steroid hormones, or whether cholesterol esterified with fatty acids other

than arachidonic acid can not serve as a precursor of the hormones, since cholesterol esterified with fatty acids other than arachidonic acid also decreases following administration of ACTH, although its rate of decrease is significantly smaller than that of cholesteryl arachidonate.

The difference in serum corticosterone levels between animals fed pyridoxine supplemented fat diet and pyridoxine-deficient fat diet in the resting state or following a single administration of ACTH was much less striking. However, on the fourth day of successive administration of ACTH, the difference became distinct. At the same time, in animals fed pyridoxine-deficient fat diet adrenal cholesterol ester arachidonic acid decreased sharply in spite of unchanged total arachidonic acid content. It is apparent that pyridoxine affects adrenocortical function through lipid metabolism.

In previous studies of the role of EFA, KUNKEL and WILLIAMS first noted an increase in cytochrome and choline oxidases in EFA deficiency. TULPULE and PATWARDHAN reported that the deficiency resulted in a reduction of glutamic, succinic and butyric acid dehydrogenase titers. KLEIN and JOHNSON first suggested that the deficiency brought about an uncoupling of oxidation from phosphorylation in mitochondria. This proposal was later supported by the works of TULPULE and WILLIAMS with homogenates and LEVIN et al. with mitochondria.

TULPULE and PATWARDHAN also observed a reduction in liver glutamic dehydrogenase activity of vitamin B_6-deficient rats, which was further decreased by a double deficiency of EFA and vitamin B_6. TULPULE and WILLIAMS suggested that this vitamin was necessary for maintaining the phosphate esterification system associated with the oxidation of reduced cytochrome C. YAMADA et al. observed that vitamin B_6 phosphate was necessary for decarboxylation from pantothenate to pantotheine, so that this vitamin directly related

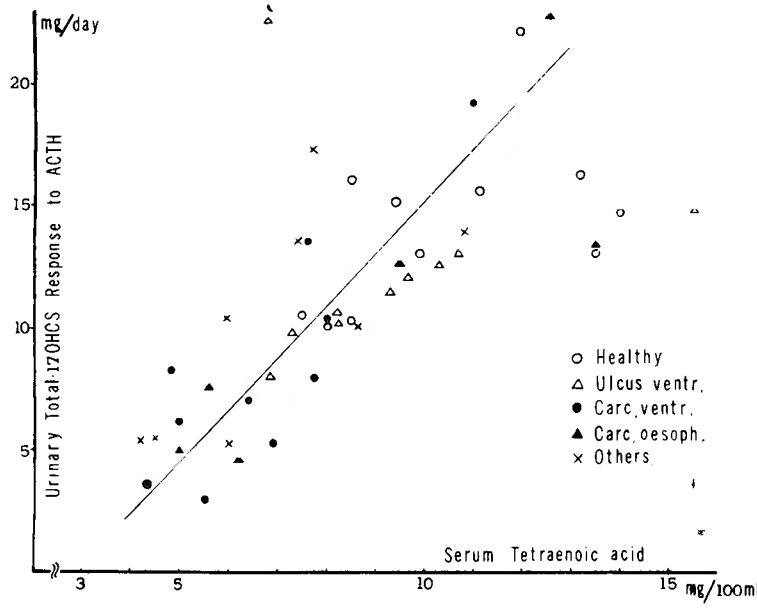

Fig. 8 The relationship between serum tetraenoic acid levels and total 17-OHCS response in urine in ACTH-test (*Fukuda*)

to Co A formation.

Thus, it is obvious that the deficiency of EFA or vitamin B_6 affects many enzyme systems and induces disturbance of lipid metabolism. It is quite plausible that in animals deficient in EFA or vitamin B_6, the formation of cholesteryl arachidonate in adrenals or the supply of materials necessary for it from plasma might be disturbed, and in EFA-deficient animals even in the resting state, and in vitamin B_6-deficient EFA supplemented animals under various stresses, their adrenals can not respond to an increased demand for adrenocortical hormones in the body.

As to the role of EFA on adrenocortical activity in man, no conclusion can be drown from present study. However, FUKUDA showed in his clinical study that there was an intimate relationship between tetraenoic acid concentration in serum and total 17-OHCS response in urine after ACTH stimulation (Fig. 8). It may be presumed that EFA play the same role in the human adrenal as in the rat.

CONCLUSION

1) The adrenals of EFA-deficient rats had lower levels of 18 : 2, 20 : 4 and 22 : 4 acids and higher levels of 20 : 3 and 22 : 3 acids both in total lipids and in cholesterol esters than those of supplemented controls.

2) Pattern of the adrenal fatty acids of the pyridoxine-deficient EFA supplemented rats was similar to that of pyridoxine and EFA supplemented controls in the resting state, but after daily administration of ACTH for 4 days the amounts of EFA *i. e.* 18 : 2, 20 : 4 and 22 : 4 acids in cholesterol esters decreased markedly.

3) Rats fed EFA-deficient diet or pyridoxine-deficient EFA supplemented diet showed reduced secretion of corticosterone by adrenal cortex ; the former even at rest, the latter after daily administration of ACTH for 4 days, as compared with the supplemented controls.

4) Adrenal total and esterified cholesterol content of the rat adrenals fed EFA-deficient diet was higher than that of supplemented controls, therefore the adrenal cholesterol content by itself is not an index of adrenocortical activity.

5) In the three diet groups, in the resting state, or following single and successive administration of ACTH, secretion of corticosterone changed parallel with cholesterol ester arachidonic acid content in the adrenals.

6) Two hours after a single injection of ACTH, when the serum corticosterone levels reached their maximum, cholesterol esterified with arachidonic acid (cholesteryl arachidonate) decreased significantly more than that esterified with other fatty acids.

7) From these observations and the fact that adrenal cholesterol is the major mother substance of adrenocortical hormones, it may be concluded that cholesteryl arachidonate is the main precursor of adrenocortical hormones in rats.

The author wishes to express his sincere gratitude to Dr. Y. HIKASA, the Assistant Professor of our clinic, for his helpful suggestion and kind guidance in the course of the work.

The gist of the present study was reported at the 36th Annual Meeting of the Japan Endocrinological Society (April, 1963), and at the 3rd Meeting of the Proceedings of Symposium on Chemical Physiology and Pathology (November, 1963).

REFERENCES

1) Aaes-Jørgensen, E. : Essential fatty acids. Physiol. Rev., **41** : 1, 1961.
2) Abell, L. L. et al. : A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem., **195** : 357, 1952.
3) Avigan, J. and Steinberg, D. : Effects of saturated and unsaturated fat on cholesterol metabolism. Proc. Soc. Exptl. Biol. Med., **97** : 814, 1958.
4) Burr, G. O. and Burr, M. M. : A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., **82** : 203, 1929.
5) Dailey, R. E. et al. : Adrenal cholesterol ester fatty acid composition of different species. Proc. Soc. Exptl. Biol. Med., **105** : 1, 1960.
6) Dailey, R. E., Swell, L. and Treadwell, C. R. : Utilization of free and esterified cholesterol-4-C^{14} for corticoid biosynthesis by hog adrenal homogenates. Proc. Soc. Exptl. Biol. Med., **110** : 571, 1962.
7) Deuel, H. J. : Nutritional significance of the fats. Progress in the Chemistry of Fats and other Lipids.. **2** : Pergamon Press, 1954.
8) Eberhagen, D., Lindlar, F. and Zöllner, N. : Über die Fettsäuren in den Lipoiden menschlicher Nebennieren. Zeitschrift für die gesamte experimentelle Medizin, **137** : 447, 1963.
9) Eisenstein, A. B. Steroid secretion by the isolated rat adrenal. Proc. Soc. Exptl. Biol. Med., **91** : 657, 1956.
10) Fukuda, H. : unpublished.
11) Fulco, A. J. and Mead, J. F. : Metabolism of essential fatty acids. VIII. Origin of 5, 8, 11-eicosatrienoic acid in the fat-deficient rat. J. Biol. Chem., **234** : 1411, 1959.
12) Fulco, A. J. and Mead, J. F. : Metabolism of esential fatty acids. IV. The biosynthesis of the octadecadienoic acids of the rat. J. Biol. Chem., **235** : 3379, 1960.
13) Funch, J. P., Jart, Aa. and Dam, H. : The effects of diets with no fat or with hydrogenated or unhydrogenated fat on grouth and tissue pathology in rats. Brit. J. Nutr., **14** : 171, 1960.
14) Hayashida, T. and Portman, O. W. : Effects of essential fatty acid deficiency on rat adrenal composition and secretory activity. Am. J. Physiol., **197** : 893, 1959.
15) Hayes, R., Savard, K. and Dorfman, R. I. : The action of adrenocorticotrophic hormone on beef adrenal slices. J. Biol. Chem., **207** : 925, 1954.
16) Haynes, R. C. : The activation of adrenal phosphorylase by the adrenocorticotropic hormone. J. Biol. Chem., **233** : 1220, 1958.
17) Hechter, O. et al. : Transformation of cholesterol and acetate to adrenal cotical hormones. Arch. Biochem. Biophys., **46** : 201, 1953.
18) Hechter, O. and Pincus, G. : Genesis of the adrenocortical secretion. Physiol. Rev., **34** : 459, 1954.
19) Hikasa, Y. et al. : Nutritional effect of fat. Sogo-Igaku, **19** : 95, 1962.
20) Hikasa, Y. et al. : Practical application of gas-iquid chromatographic analysis of fatty acids. Sushin-Igaku, **18** : 921, 1963.
21) Hikasa, Y. et al. : Roles of EFA on adrenocortical capacity. Nihon- Rinsho, **22** : 142, 1964.
22) Hikasa, Y. et al. : Effects of essential fatty acids and vitamin B_6 upon adrenocortical secretory activity. Procedings of Symposium on Chemical Physiology and Pathology, **3** : 89. 1963.
23) Hirano, Y. : unpublished.
24) Hirsch, J. and Ahrens, H. : The separation of complex lipid mixtures by the use of silicic acid chromatography. J. Biol. Chem., **233** : 311, 1958.
25) Holman, R. T. and Hayes, H. : Determination of polyunsaturated acids in lipides of plasma and tissue. Anal. Chem., **30** : 1422, 1958.
26) Howton, D. R. and Mead, J. F. : Metabolism of essential fatty acids. X. Conversion of 8, 11, 14-eicosatrienoic acid to arachidonic acid in the rat. J. Biol. Chem., **235** : 3385, 1960.
27) Hurley, L. S. and Mackenzie, J. B. : Adrenal function in the pantothenic acid-deficient rat ; liver glycogen, blood glucose, adrenal cholesterol and adrenal ascorbic acid levels. J. Nutr., **54** : 403, 1954.
28) Ishihara, N. : Corticoid content in blood of the vitamin B_6 deficiency rat and biohistology of the adrenal gland. Vitamin, **18** : 31, 1959.
29) Ishimaru, H. : Electron microscopic study of the adrenal cortex especially the influence of essential fatty acid deficiency on adrenocortical structure. Arch. Jap. Chir., **31** : 536, 1962.
30) James, A. T. : Qualitative and quantitative determination of fatty acids by gas-liquid chromatography. Methods

of Biochemical Analysis, 8 : 1, 1960.
31) Jindo, A. : A micromethod for determining polyunsaturated fatty acids ; Its clinical and experimental applications. Arch. Jap. Chir., **30** : 1, 1961.
32) Kirschman, J. C. and Coniglio, J. G. : The role of pyridoxine in the metabolism of polyunsaturated fatty acids in rats. J. Biol. Chem., **236** : 2200, 1961.
33) Klein, P. D. and Johnson, R. M. : Phosphorus metabolism in unsaturated fatty acid deficient rats. J. Biol. Chem., **211** : 103, 1951.
34) Klein, P. D. and Johnson, R. M. : A study of the onset of unsaturated fatty acid deficiency in subceller particles of rat livers. Arch. Biochem. Biophys., **48** : 380, 1954.
35) Klein, P. D. and Dahl, R. M. : The sensitivity of cholesterol esterification to enviromental stress. J. Biol. Chem., **236** : 1658, 1961.
36) Kumano, M. : Experimental studies on the effect of administration of essential fatty acids upon adrenocortical capacity from the view-point of cholesterol metabolism. Arch. Jap. Chir., **31** : 115, 1962.
37) Kunkel, H. O. and Williams, J. N. : The effects of fat deficiency upon enzyme activity in the rat. J. Biol. Chem., **189** : 755, 1951.
38) Levin, E., Johnson, R. M. and Albert, S. : Mitochondrial changes associated with essential fatty acid deficiency in rats. J. Biol. Chem., **228** : 15, 1957.
39) Long, C. N. H. : The conditions associated with the secretion of the adrenal cortex. Fed. Proc., **6** : 461, 1947.
40) Lossow, W. J., Brot, N. and Chaikoff, I. L. : Disposition of the cholesterol moiety of a chylomicron-containing lipoprotein fraction of chyle in the rat. J. Lipid Res., **3** : 207, 1962.
41) Lynn, W. S., Staple, E. and Gurin, S. : Catabolism of cholesterol by *in vitro* systems. Fed. Proc., **14** : 783, 1955.
42) Machlin, L. J. : Effect of dietary linolenate on the proportion of linoleate and arachidonate in liver fat. Nature, **194** : 868, 1962.
43) Marco, G. et al. : Dietary effects of fats upon fatty acid composition of the mitochondria. Arch. Biochem. Biophys., **94** : 115, 1961.
44) Maruyama, I. : Effect of essential fatty acids and pyridoxine on the formation of gallstones, especially cholesterol stones. Arch. Jap. Chir., **34** : 19, 1965.
45) Matsuda, S. : Experimental study on the nutritional significance of fat from the view-point of effects on the liver glycogen content. Arch. Jap. Chir., **8** : 3008, 1959.
46) Mead, J. F. et al. : Metabolism of essential fatty acids. Incorporation of acetate into arachidonic acid. J. Biol. Chem., **205** : 683, 1953.
47) Mead, J. F. and Slaton, W. H. : Metabolism of essential fatty acids. Ⅲ. Isolation of 5, 8, 11-eicosatrienoic acid from fat-deficient rats. J. Biol. Chem., **219** : 705, 1956.
48) Mead, J. F. : The metabolism of the essential fatty acids. Ⅵ. Distribution of unsaturated fatty acids in rats on fat-free and supplemented diets. J. Biol. Chem., **227** (2) : 1025, 1957.
49) Mead, J. F. and Howton, D. R. : Metabolism of essential fatty acids. Ⅶ. Conversion of γ-linolenic acid to arachidonic acid. J. Biol. Chem., **221** (2) : 575, 1957.
50) Mohrhauer, H. and Holman, R. T. : Effect of linolenic acid upon the metabolism of linoleic acid. J. Nutr., **81** : 67, 1963.
51) Mohrhauer, H. and Holman, R. T. : The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. J. Lipid Res., **4** : 151, 1963.
52) Nagase, M. : Experimental study on pathogenesis of acute postoperative pulmonary edema. Arch. Jap. Chir., **29** : 69, 1960.
53) Nagase, M., Ishimaru, H. and Muraoka, R. : Effects of lipids on adrenocortical function. Saishin-Igaku, **17** : 941, 1962.
54) Peluffo, R. O., Brenner, R. R. and Mercuri, O. : Action of linoleic and arachidonic acids upon the eicosatrienoic acid level in rat heart and liver. J. Nutr., **81** : 110, 1963.
55) Reiss, M. et al. : The *in vitro* action of ACTH on the oxygen consumption of slices of cattle adrenal cortex. J. Endocrin., **9** : 379, 1953.
56) Saba, N. and Hechter, O. : Cholesterol-4-C^{14} metabolism in adrenal homogenates. Fed. Proc., **14** : 775, 1955.
57) Saffran, M. and Bayliss, M. J. : *In vitro* bioassay of corticotrophin. Endocrinology, **52** : 140, 1953.
58) Sayers, G. et al. : Effect of adrenotropic hormone on ascorbic acid and cholesterol content of the adrenal. Proc. Soc. Exptl. Biol., **55** : 238, 1941.

59) Sinclair, H. M. : Essential fatty acids. Fourth International Conference on Biochemical Problems of Lipids, p. 219, London, Butterworths, 1958.
60) Skovstead, L., Funch, J. P. and Dam, H. : *In vivo* stimulation by adrenocorticotrophin (ACTH) of rat adrenals in relatoin to dietary fat. J. Endocrin., **26** : 457, 1963.
61) Smith, J. and DeLuca, H. F. : Essential fatty acid deficiency and rat liver homogenate oxidations. J. Nutr., **79** : 416, 1963.
62) Staple, E., Lynn, W. and Gurin, S. : An enzymatic cleavage of the cholesterol side chain. J. Biol. Chem., **219** : 845, 1956.
63) Steinberg, G. et al. Metabolism of essential fatty acids. IV. Incorporation of linoleate into arachidonic acid. J. Biol. Chem., **220** : 257, 1956.
64) Steinberg, G. et al. : Metabolism of essential fatty acids. V. Metabolic pathway of linolenic acid. J. Biol. Chem., **224** : 1957.
65) Swell, L. et al. : Tissue lipid fatty acid composition in pyridoxine deficient rats. J. Nutr., **74** : 148, 1961.
66) Tamaki, Y. : Experimental study on the effect of essential fatty acid deficiency on adrenocortical function. Arch. Jap. Chir., **30** : 611, 1961.
67) Tulpule, P. G. and Patwardhan, V. N. : Effect of fat and pyridoxine deficiencies on rat liver dehydrogenases. Arch. Biochem. Biophys., **39** : 450, 1952.
68) Tulpule, P. G. and Williams, J. N. : Study of the role of eesential fatty acids in liver metabolism. J. Biol. Chem., **217** : 229, 1955.
69) Verdino, B. et al. : Metabolism of 4, 7, 10, 13, 16-docosapentaenoic acid in the essential fatty acid deficient rat. J. Nutr., **83** : 234, 1961.
70) Vogt, M. : Cortical secretion of the isolated perfused adrenal. J. Physiol., **113** : 129, 1951.
71) Werbin, H. and LeRoy, G. V. : Cholesterol—a precursor of tetrahydrocorticosterone in man. J. Am. Chem. Soc., **76** : 5260, 1951.
72) Werbin, H. and LeRoy, G. V. : Cholesternl—a precursor of tetrahydrohydrocortisone (TEF) and 11-ketoetiocholanolone in man. Fed. Proc., **14** : 303, 1955.
73) Werbin, H. and Chaikoff, I. L. : Utilization of adrenal gland cholesterol for synthesis of cortisol by the intact normal and the ACTH-treated guinea pig. Arch. Biochem. Biophys., **93** : 476, 1961.
74) Williams, M. A. and Scheier, G. E. : Effect of methyl arachidonate supplementation on fatty acid composition of liver of pyridoxine deficient rats. J. Nutr., **74** : 9, 1961.
75) Yamada, K., Sawaki, S. and Hayami, S. : Participation of vitamin B_6 in the biosynthesis of Coenzyme A. J. Vitaminol., **2** : 296, 1956.
76) Yoshinaga, M. : Experimental studies on the initiating factor of cholesterol gallstones, especially on the influence of essential fatty acids and pyridoxine on the bile constituents. Arch. Jap. Chir., **34** : 1, 1965.
77) Zaffaroni, A., Hechter, O. and Pincus, G. : Adrenal conversion of C^{14} labeled cholesterol and acetate to adrenal cortical hormones. J. Am. Chem. Soc., **73** : 1390, 1951.

和文抄録

副腎皮質機能に占める不可欠脂酸ならびにピリドキシンの役割に関する実験的研究

京都大学医学部外科学教室第2講座（指導：木村忠司教授）

村　岡　隆　介

従来より教室では脂質殊に不可欠脂酸の生理学的意義の解明に努めて来たが，その一環として，脂質殊に不可欠脂酸と副腎皮質機能との関係をも検討し，不可欠脂酸の欠乏は副腎皮質機能の障害を招来することを実験的並びに臨床的に確認するに至つた．

本研究は脂質殊に不可欠脂酸の副腎皮質機能に対する作用機序をうかがう目的で，脂質食リノール酸を約50%の割合に含有する精製ゴマ油を15%含む，脂質欠乏食（精製ゴマ油を澱粉で置換）及び脂質代謝に重要な関係を有するビタミンB_6を欠乏せしめたビタミンB_6欠乏脂質食で飼育せる3群の白鼠について，ACTH 1回及び4回連続負荷を行つて，副腎脂質と副腎皮質機能の関係を検討し，併せて剖検及び手術により得られた人副腎の脂質分析を行つた．副腎脂質よりのコレステロールエステル画分々離には Silicic acid chromatography を用い，脂酸分析には従来の Alkaline isomerization 法に Gas-liquid chromatography を併用して，副腎総脂質及びコレステロールエステル画分について夫々コレステロール及び脂酸の分析を行い，次の結果を得た．尚血清中のコルチコステロンの測定は共同研究者福田が行つた．

1）上記3群とも不可欠脂酸を含めて不飽和脂酸はコレステロールエステル画分中に大半が含有され，脂質欠乏食餌で飼育せる試獣では，リノール酸，アラキドン酸及びDocosatetraenoic acid の含量が著しく減少し，Eicosatrienoic acid 及び Docosatrienoic acid のそれが増加しており，その副腎皮質機能は ACTH 1回並びに4回負荷時には勿論すでに安静時に於ても強く障害されている．

2）ビタミンB_6欠乏脂質食群の副腎では，安静時にはビタミンB_6を充分に投与した脂質食群と同程度か或はそれ以上の不可欠脂酸を含有し，副腎皮質機能も安静時及び ACTH 1回負荷時には対照脂質食群に比して殆ど遜色を示さないが，ACTH 連続4回負荷では，副腎総脂質中には対照脂質食群と同程度の不可欠脂酸を含有するにも拘らず，コレステロールエステル画分中の不可欠脂酸は著しく減少して，むしろ脂質欠乏食群のそれに近づき，同時に副腎皮質機能も強く障害されて脂質欠乏食群のそれに近づく．

3）これら3群白鼠の血中コルチコステロン濃度は，安静時並びに ACTH 負荷時とも副腎コレステロールエステル画分中に含有される不可欠脂酸就中アラキドン酸の量とよく平行的関係を示す．

4）副腎のコレステロールはステロイドホルモンの Precursorたり得るものであるが，3群ともその大部分（約90%）がエステル型であつて，ACTH 負荷により急速に減少する．しかし乍ら副腎のコレステロール含量は副腎皮質機能の低下している脂質欠乏食群に於て最も高く，従つてコレステロールの含量それ自体は副腎皮質機能と平行的関係を示さない．

5）ACTH 1回負荷に際して，血清コルチコステロン濃度がほぼ最高値を示す負荷後2時間目の副腎コレステロールエステル脂酸のうち，Gas-liquid chromatographyによれば，3群ともアラキドン酸の占める百分率のみが特異的に有意の減少を示す．即ち ACTH 負荷によつてアラキドン酸とエステル結合しているコレステロール（Cholesteryl arachidonate）が，それ以外の脂酸とエステル結合するコレステロールに比べて特異的に減少することを示すものである．

6）以上の成績から，白鼠に於ては副腎中の Cholesteryl arachidonate が副腎ステロイドホルモンの主な Precursor であると考えられる．

7）剖検或は手術により得られた5例の人副腎のコレステロール及び脂酸を分析したが，人副腎の脂酸構成は白鼠に比べて Eicosatrienoic acid が多量に含有され，アラキドン酸は比較的少ない．しかし乍ら共同研究者福田が行なつた ACTH-test の際の尿中総17-OHCS反応量と血清Tetraenoic acid（殆どがアラキドン酸）の間には密接な相関々係が存在し，人に於てもアラキドン酸が白鼠に於けると同様の意義を有するものと憶測される．